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Biosafety Evaluation of Transgenic Potatoes: Gene Flow from Transgenic Potatoes

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ABSTRACT

A potential concern associated with the release of transgenic crops is the risk of gene flow to neighboring crops of the same species or to related species. In potato there is little accurate information or experience on effective pollination distances for gene flow between plants. The incidence of intraspecific or interspecific pollination as a basis for gene flow has only received minimal attention since true seed production is unimportant for plant propagation. Gene flow of transgenes from potato crops can arise from several different paths. The most obvious is from true seeds arising on neighboring non-genetically modified (GM) plants, either potatoes or other *Solanum* species, following pollen dispersal from transgenic potato crops. Alternatively, it may arise from true seeds developing on the transgenic potato crops which accumulate in the seed bank for germination in future years. This may arise from either self pollination within transgenic potato crops or pollen dispersal from neighboring non-transgenic potato crops or other *Solanum* species. Germination of such seed may occur many years later, and the resulting plants may contribute to transgene flow *via* either seed or pollen dispersal. In a similar manner, gene flow

may arise from volunteer plants arising in the season following the transgenic potato crop from tubers remaining after harvest. This paper summarizes information relevant to these pathways for gene flow from transgenic potato crops and discusses approaches for minimizing such gene flows.

Key words: Transgenic potato; GM potato; Gene flow; Hybridization; Isolation distances.

INTRODUCTION

The release of genetically modified (GM) crops into agricultural production has raised considerable debate, especially among the general public, politicians, and bureaucrats (Nap *et al.*, 2003). A main concern associated with the release of transgenic crops is the risk of gene flow from transgenic crops to neighboring crops of the same species or related species (Conner *et al.*, 2003). This may undermine the containment of transgenes and compromise the segregation of transgenic and non-transgenic crops. A common concern raised about gene flow involving transgenes is the potential for the resulting plants to become agricultural weeds or invade natural habitats and compromise natural biodiversity (Conner *et al.*, 2003).

Many decades of experience in seed production of specific cultivars in many crops have defined well-established isolation distances that are rigidly enforced to maintain seed purity to defined legal specifications (Frankel and Galum, 1977). However, true seed production is unimportant in asexually propagated crops such as potatoes. Consequently there is very little accurate information or experience on effective pollination distances in potato and isolation required to mitigate intraspecific or interspecific gene flows (Conner, 1994). Such information is essential for regulatory

authorities to set realistic isolation distances and management strategies to contain gene flow from transgenic potato crops.

This paper summarizes the potential pathways by which gene flow can occur from transgenic potato crops to neighboring non-transgenic potato crops or other *Solanum* species. The incidence of such gene flow is discussed along with various approaches that can be used to minimize or prevent such events.

POLLEN-MEDIATED GENE FLOW FROM FIELD-GROWN TRANSGENIC POTATOES

The total number of field tests on transgenic potatoes is estimated to have exceeded 2000 over the last 20 years (Conner 2006). However, the incidence of pollen-mediated transgene dispersal has only been reported from a small number of these field tests. This represents a wasted opportunity, and more effort in recording and reporting this data would have greatly increased the confidence in the conclusions made to date and facilitate future applications for field testing transgenic potatoes (Conner 2006).

Pollen-mediated transgene dispersal has been investigated by estimating the frequency of transgenic progeny produced on non-transgenic pollen-trap cultivars planted at varying distances surrounding the transgenic plots. In such studies, synchronized flowering times for the transgenic and pollen-trap plants was ensured by removing flower buds from the pollen-trap when necessary. The majority of these independently performed studies have reported very similar results, with very limited dispersal of pollen over short distances from the edge of transgenic potatoes. The frequency of transgenic progeny from non-transgenic potatoes growing in rows

adjacent to transgenic potatoes ranged from 1% to 24% and rapidly fell to negligible levels within 3~5 m (Tynan *et al.*, 1990; Dale *et al.*, 1992; Conner, 1993, 1995; McPartlan and Dale, 1994). One study concluded that pollen-mediated transgene dispersal can occur at high frequencies up to 1000 m from field trials of transgenic potato (Skogsmyr, 1994). However, this latter study has been discredited and considered to be based on a high frequency of false positives rather than on actual gene flow (Conner and Dale, 1996). Based on the collective data it was recommended that a distance of 20 m is generally adequate for containing novel gene constructs during initial field tests of transgenic potato (Conner and Dale, 1996).

More recently, we completed another comprehensive study from seven field test sites over six seasons that screened a total of over 1.3 million progeny from non-transgenic pollen-trap buffer rows (Erasmuson *et al.*, 2005). The accuracy of this phenotypic screening was verified by PCR in a sample of the progeny. In the buffer row immediately adjacent to the field trial, the frequency of transgenic progeny ranged from 0.7 to 5.9 per 10,000 and declined to between 0 and 0.5 per 10,000 at the third buffer row from the field trial (a distance of 2.25 m). This also confirms that isolation distances of 20 m are sufficient for the initial field evaluation of transgenic potatoes containing novel genes. If such data had been repeatedly verified in many environments and for additional potato cultivars, some regulatory bodies might no longer require the removal of flower buds during field tests of transgenic potato or may have set more-realistic isolation distances (Conner 2006).

All of the gene flow studies from transgenic potato field tests have only involved small-scale field tests. Such field tests may often involve transgenic lines, some of which exhibit poor agronomic performance, slow growth, and a failure to flower

(Conner, 2006). When commercial fields of transgenic potatoes are grown on a larger scale with lines of equal agronomic performance to the parent cultivar, the load of transgenic pollen may be considerably higher. This suggests the need to demand greater isolation distances for commercial transgenic potato fields to mitigate pollen-mediated gene flow. However, anecdotal information from potato breeders and farmers suggests that pollen dispersal in commercial fields also only occurs over short distances. Berry formation on male-sterile potato cultivars is only occasionally observed on plants growing immediately alongside male-fertile cultivars, suggesting that effective pollen dispersal in potato only occurs over a few meters (Conner and Dale, 1996).

VOLUNTEER POTATOES AS SOURCE OF GENE FLOW

In an agricultural context potatoes often appear as "weeds" in the same field or neighboring areas in seasons following a potato crop. These may arise from true seeds or from tubers remaining in the ground after harvest. Both sexual and asexual propagation can therefore produce transgenic potato plants in subsequent seasons from which gene flow pathways can arise in later years.

The number of true seeds produced in commercial fields of fertile potato cultivars is highly dependent upon the cultivar grown, environmental conditions, and pest/pathogen activity, but can be as high as 150~250 million per hectare (Accatino 1980; Lawson 1983). Seeds may remain dormant in the soil for up to 2 years and have been reported to retain viability over a seven-year rotation. Consequently, true potato seeds have the potential to become a weed problem in subsequent crops (Lawson 1983). Furthermore, the potato berries can be consumed by various vertebrates which can provide a vector for seed dispersal beyond the field where the

potato crop was grown. Germination of such transgenic seed and potential establishment of plants can provide a pathway for gene flow. Management practices to minimize such gene flow pathways may be difficult to implement because germination from the seed bank is unpredictable and may occur many years later and/or at sites other than that at which the seed was dispersed.

Potato plants as weeds can also arise from the inability to remove all tubers from a field during harvest, resulting in the appearance of volunteer (ground keeper or self-set) plants the following year. Up to 367,000 tubers per hectare have been estimated to remain in the field after mechanical harvest (Lutman, 1977). Depending of the severity of the winter, many of these tubers will remain viable until the next spring. Potato tubers require 50 frost-hour equivalents at -2 ºC or below to kill tubers, e.g., 25 h at -2 ºC, or 5 h at -10 ºC, etc. (Lumkes and Sijtsma, 1972). In the temperate British climate, up to 80% of tubers left after harvest died in even mild winters (Lutman, 1977). The remaining viable volunteer tubers do not remain dormant and will always sprout the next season.

The incidence of volunteer plants following the growing of transgenic potatoes has only been reported from a small number of field tests from New Zealand (Conner, 1993, 1995; Reader *et al.*, 2005). Even when transgenic potatoes are carefully hand-harvested, the frequency of volunteers ranged from 0.4/m2 to 2.9/m2. Transgenic volunteer potatoes appeared less frequently than volunteers of the non-transgenic parental cultivars. This was consistent when calculated on a relative basis to both the number of plants in the field trial and the number of harvested tubers. Therefore, in small-scale field trials, transgenic potato lines are, on average, likely to be less invasive than the non-transgenic parental cultivars. This is not unexpected since

many transgenic lines in such small-scale field tests exhibit poor agronomic performance and lower tuber yields (Conner, 2006). However, when commercial fields of transgenic potatoes are grown with transgenic lines of equal agronomic performance to the parent cultivar, the frequency of volunteers is likely to be similar for transgenic and non-transgenic cultivars.

Despite the potential for volunteer potatoes to become a weed problem, they do not usually persist for more than one or two seasons and are rarely seen outside cultivated fields because they do not have the invasive potential of most weeds (Conner *et al.*, 1997). The expression of transgenes is not anticipated to increase the invasiveness of potato cultivars any more than the traditional breeding for traits such as resistance to pests, diseases, drought, and frost (Conner *et al.*, 1997). However, the presence of such volunteers provides a potential pathway for gene flow in seasons following the transgenic potato crop.

INTERSPECIFIC TRANSGENE FLOW TO OTHER SOLANUM SPECIES

The potential of gene flow from transgenic crops to other related species is commonly raised as a concern. The introgression of transgenes into other species may increase the survival and spread of weeds that may invade agricultural land or natural habitats and may impact natural biodiversity (Conner *et al.*, 2003). Natural gene flow between cultivated potato crops and related *Solanum* species *via* hybrid swarms is well recognized in the Peruvian/Bolivian center of origin for the potato (Ross, 1986), where 130 wild potato species occur (Spooner and Hijmans, 2001). As part of a biosafety assessment for transgenic potato releases in this region, interspecific hybridization was confirmed between potato and six species found close

to potato plots (Celis *et al.*, 2004). The recovery of hybrids between these species (*S. albicans, S. acaule, S. chomathophilum, S. raphanifolium, S. bukasovii, and S. sparsipilum*) were all confirmed through AFLP markers. Spontaneous field hybridization was also confirmed over short distances from a potato pollen source and between potato and *S. acaule, S. bukasovii,* and *S. sparsipilum.*

In most other regions of the world, outside the center of origin for potato, the potential of spontaneous hybridization between potato and other *Solanum* species is substantially less. However it is important to consider the species that grow in the region where transgenic potatoes are to be cultivated. In temperate regions investigations have focused on the potential for gene transfer to *S. nigrum* and related nightshade species since these are commonly found in and around potato fields. Sterile F1 hybrids have been reported between potato and black nightshade, but only after resorting to very early embryo rescue and *in vitro* culture of the embryos (Eijlander and Stiekema, 1994). The resulting interspecific hybrids were very weak and difficult to keep alive. Similar attempts to make this *S. tuberosum* x *S. nigrum* cross elsewhere have failed (Dale, 1993). Other attempts to hybridize *S. dulcamara* and *S. tuberosum* have also failed (Eijlander and Stiekema, 1994; Dale 1993). Gene transfer from transgenic potatoes to black nightshade was monitored during one of our earlier contained field trials in the 1989/1990 season. No evidence for gene transfer was obtained, despite screening over 53,000 black nightshade seedlings (Conner, 1993, 1995). Similar results using 7,600 *S. nigrum* seedlings were observed in Australia (Conner, 1994).

As part of the biosafety assessment for field trials on transgenic potatoes in New Zealand, we have investigated the potential for hybridization *via* hand pollination

between three potato cultivars and a range of *Solanum* species found in New Zealand. These included the three indigenous species (*S. americanum, S. aviculare,* and *S. laciniatum*) and ten exotic or garden species (*S. brevidens, S. muricatum, S. nigrum, S. furcatum, S. chenopodioides, S. dulcamara, S. jasminoides, S. physalifolium, S. pseudocapsicum*, and *S. melongena*). All attempted interspecific crosses failed to produce seed, whereas control intragenic crosses were successful for all species except *S. jasminoides* (A. J. Conner *et al.*, unpublished data).

When spontaneous interspecific hybridization between potato and related species is possible, gene flow can occur in either of two directions. The most obvious is from hybrid seed formation on neighboring *Solanum* species, following pollen dispersal from transgenic potato crops. Alternatively, interspecific hybrid seeds may develop on the transgenic potato crops following pollen dispersal from neighboring *Solanum* species. Such hybrid seed may accumulate in the seed bank for germination in future years. Germination of such seed may occur many years later, and the resulting hybrid plants may contribute to transgene introgression into other *Solanum* species by further backcrossing.

MITIGATING GENE FLOW FROM TRANSGENIC POTATOES

An approach often suggested to mitigate gene flow from transgenic potatoes involves the use of male sterility. Some existing potatoes cultivars are already male sterile. For example, the Andean cultivar "Revolucion" has been used as a parental cultivar in the transgenic development of nematode resistance for proposed release in the Peruvian/Bolivian center of diversity of potato (Celis *et al.*, 2004). Alternatively, male sterility in potato can be derived through transgenic development by targeting pollen expression of a suicide gene such as barnase (citations to be inserted).

However, this approach only targets mitigation of pollen-mediated gene flow from transgenic potatoes to neighboring non-transgenic potatoes or related *Solanum* species. The use of male-sterile potatoes does not eliminate hybridization with pollen from neighboring non-transgenic potatoes or related *Solanum* species. The resulting true seed can accumulate in the seed bank and contribute to gene flow to non-transgenic potatoes or introgression into related species following germination in later years.

To completely eliminate the possibility of gene flow would require the development of non-flowering genotypes. In most crops, this would not be feasible since flowering is an essential prerequisite for either grain or fruit production, as well as seed propagation. However, for potatoes, flowering is not required for either plant propagation or the harvested produce. In an attempt to recover non-flowering lines of important New Zealand potatoes cultivars we field-screened large numbers of plant lines regenerated from cell culture. While several non-flowering somaclonal variants were identified, these lines unfortunately also possessed a range of poor attributes (A.J. Conner *et al.*, unpublished data). Further screening might be successful. Another approach might be to design a transgenic strategy to knock-out flowering.

An approach has recently been described for eliminating transgenes during pollen development. This involves the recombinase-mediated auto-excision of transgenes controlled by a tightly regulated microspore-specific promoter to remove either selectable marker genes or all of the transgenes during pollen development (Mlynárová *et al.*, 2006). This provides a system to mitigate gene flow *via* pollen dispersal and could provide a valuable containment system for the coexistence of GM and non-GM agriculture. This is a convenient approach in potatoes since they

are clonally propagated and transgenic pollen formation is unnecessary for cultivar propagation. A similar approach could also be taken to effect transgene excision from ovules and mitigate sexual transmission and gene flow of transgenes.

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